Molecular and Cellular Endocrinology 336 (2011) 162–168

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

How does cAMP/protein kinase A signaling lead to tumors in the adrenal cortex and other tissues?

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ARTICLE INFO

Article history:
Received 4 October 2010
Received in revised form
15 November 2010
Accepted 15 November 2010

Keywords:
Adrenal cortex
Protein kinase A
Primary pigmented nodular adrenocortical disease
ACTH-independent macronodular adrenal hyperplasia
Adrenal tumor
Phosphodiesterase
Wnt signaling

ABSTRACT

The overwhelming majority of benign lesions of the adrenal cortex leading to Cushing syndrome are linked to one or another abnormality of the cAMP signaling pathway. A small number of both massive macronodular adrenocortical disease and cortisol-producing adenomas harbor somatic GNAS mutations. Micronodular adrenocortical hyperplasias are either pigmented (the classic form being that of primary pigmented nodular adrenocortical disease) or non-pigmented; micronodular adrenocortical hyperplasias can be seen in the context of other conditions or isolated; for example, primary pigmented nodular adrenocortical disease usually occurs in the context of Carney complex, but isolated primary pigmented nodular adrenocortical disease has also been described. Both Carney complex and isolated primary pigmented nodular adrenocortical disease are caused by germline *PRKAR1A* mutations; somatic mutations of this gene that regulates cAMP-dependent protein kinase are also found in cortisol-producing adenomas, and abnormalities of PKA are present in most cases of massive macronodular adrenocortical disease. Micronodular adrenocortical hyperplasias and some cortisol-producing adenomas are associated with phosphodiesterase 11A and 8B defects, coded, respectively, by the *PDE11A* and *PDE8B* genes. Mouse models of *Prkar1a* deficiency also show that increased cAMP signaling leads to tumors in adrenal cortex and other tissues. In this review, we summarize all recent data from ours and other laboratories, supporting the view that *Wnt*-signaling acts as an important mediator of tumorigenicity induced by abnormal PRKAR1A function and aberrant cAMP signaling.

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1. Introduction

Cyclic adenosine monophosphate (cAMP) is produced following activation of adenylate cyclase downstream of G protein-coupled receptors. cAMP-dependent protein kinase (PKA), a serine/theronine kinase, is the main mediator of cAMP signaling, a ubiquitous signaling pathway that is conserved in all eukaryotes (Bossis and Stratakis, 2004). The PKA holoenzyme is a hetero-tetramer composed of two regulatory subunits (usually, but not

Abbreviations: cAMP, cyclic adenosine monophosphate; CNC, Carney complex; CS, Cushing syndrome; CREB, cAMP response element-binding; MAH, micronodular adrenocortical hyperplasia; PDE, phosphodiesterases; PKA, protein kinase A; PPNAD, primary pigmented nodular adrenocortical disease; RIα, 1-α regulatory.

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0303-7207/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.mce.2010.11.018

Table 1
Types of micronodular adrenocortical disease/hyperplasia (associated with multiple adrenocortical nodules, mostly less than 1 cm in diameter).

Disease	Epidemiology	Description	Genes
iPPNAD	Children and young adults	Microadenomatous hyperplasia with at least some pigment	*PRKAR1A*, *PDE11A*, *PDE8B*, 2p16, others
cPPNAD	Children, young and middle aged adults with CNC	Microadenomatous hyperplasia with pigmentation and (mostly) internodular atrophy	*PRKAR1A*, 2p16, others
MAH	Mostly children and young adults	Microadenomatous hyperplasia with internodular hyperplasia and limited or absent pigment	*PDE11A*, 2p12–p16, 5q, others

CNC, Carney complex; iPPNAD, isolated primary pigmented nodular adrenocortical disease; C-PPNAD, primary pigmented nodular adrenocortical disease associated with Carney complex; MAH, micronodular adrenal hyperplasia; PDE, phosphodiesterase; *PDE11A*, the gene for PDE11A; *PDE8B*, the gene for PDE8B, *PRKAR1A*, protein kinase A regulatory subunit 1α.

always, identical), each bound to one catalytic subunit (Bossis and Stratakis, 2004; Skalhegg and Tasken, 2000). Four major regulatory (RIα, RIβ, RIIα, and RIIβ) and four catalytic (Cα, Cβ, Cγ and Prkx) subunits have been identified; type I PKA contains either regulatory subunit RIα or RIβ in its structure; type II PKA contains either regulatory subunit RIIα or RIIβ (Gamm et al., 1996; McKnight et al., 1988; Uhler and McKnight, 1987).

To bind and inhibit the catalytic subunit, RIα undergoes a dramatic conformational change in which the two cAMP-binding domains uncouple and wrap around the large lobe of the catalytic subunit (Kim et al., 2007). cAMP binding to the RIα subunits of PKA releases the catalytic subunits, which allows them to phosphorylate cytoplasmic targets and/or cAMP response element-binding (CREB) protein, resulting in activation of DNA transcription of cAMP-responsive element-containing genes (Bossis et al., 2004; Pearce et al., 2010). PKA system has a substantial capacity of self-regulation; over-expression of Cα or Cβ in cell culture results in significant compensation by an increase in RIα protein (Uhler and McKnight, 1987). The cellular localization of PKA has a pivotal role in determining which substrates are phosphorylated and it is controlled by the multidomain scaffolding proteins known as A-kinase anchor proteins, which bind to the R subunits of PKA hetero-tetramers (Wong and Scott, 2004).

2. Abnormalities of cAMP/PKA signaling in adrenal hyperplasias and tumors

PKA signaling has been extensively studied for many years, but only recently abnormalities of the PKA signaling pathway have been linked to tumor formation in endocrine tissues. This association was initially observed in McCune–Albright syndrome, which is caused by activating mutations in Gsα or GNAS (the product of the Gsp oncogene). *GNAS* activating mutations lead to constitutive stimulation of adenylate cyclase and PKA activation, and a variety of manifestations, including the classic triad of polyostotic fibrous dysplasia, café au lait skin pigmentation, and autonomous endocrine hyperfunction (Weinstein et al., 1991). The most frequent affected endocrine tissues are pituitary, ovarian and thyroid, but bilateral macronodular adrenocortical hyperplasia can also be found in the context of MAS (Lee et al., 1986; Stratakis and Kirschner, 1998). These and other molecular findings discussed below demonstrate the strong biological relevance of PKA activation in adrenal tumorigenesis.

The first demonstration of PKA involvement with human disease was the finding that inactivating mutations of the *PRKAR1A* gene coding for the 1-α regulatory (RIα) subunit of protein kinase A (PKA) are responsible for Carney complex (CNC) in the majority of patients (Kirschner et al., 2000a,b). CNC is a multiple neoplasia syndrome that is inherited in an autosomal dominant manner and is characterized by several types of skin tumors and pigmented lesions, myxomas, schwannomas, liver and other cancers, and endocrine neoplasms (Carney et al., 1985; Stratakis et al., 2001). In a recent review of 353 patients with CNC from 185 families, patients from all ethnicities and with a wide spectrum of clinical manifestations were described (Bertherat et al., 2009; Horvath et al., 2010; Stratakis et al., 2001). *PRKAR1A* defects were found in 73% of these patients. Primary pigmented nodular adrenocortical disease (PPNAD) was the most common endocrine tumor associated with CNC, occurring in 60% of the CNC patients (Bertherat et al., 2009). Isolated PPNAD was the only manifestation in 12% of patients carrying *PRKAR1A* defects.

Among the remaining kindreds with micronodular adrenocortical hyperplasia (MAH) and no evidence of *PRKAR1A* mutation, subgroups of patients were identified by clinical and histopathological criteria (Table 1) (Stratakis, 2009). Genetic defects in cAMP-binding phosphodiesterases (PDEs) have been described in isolated MAH. Five different *PDE11A* mutations were identified so far in patients with isolated MAH or PPNAD; three of them resulted in premature stop codon generation and the other two were single base substitutions in the catalytic domain of the protein, significantly affecting the ability of PDE11A to degrade cAMP *in vitro* (Horvath et al., 2006a,b). Two missense *PDE11A* substitutions, R804H and R867G, were also more frequent among patients with sporadic adrenocortical tumors (Horvath et al., 2006b). In addition, the chromosomal locus harboring the gene encoding phosphodiesterase 8B (PDE8B) was the second most likely region to be associated with a predisposition to isolated MAH (Horvath et al., 2006a). Sequencing of the *PDE8B*-coding regions identified a single base substitution (c.914A>T, p.H305P) in a young girl with Cushing syndrome (CS), which impaired the ability of the mutant protein to degrade cAMP (Horvath et al., 2008).

Adrenocorticotropic hormone (ACTH)-independent macronodular adrenal hyperplasia, also known as massive macronodular adrenocortical disease is a rare cause of CS (Lacroix, 2009; Zhang et al., 2009). Cortisol production in ACTH-independent macronodular adrenal hyperplasia can be regulated by the aberrant expression of G-protein-coupled receptors other than ACTH (Lacroix et al., 2001, 2004). Somatic losses of the 17q22–24 region and PKA activity changes demonstrated that cyclic (c) AMP/protein kinase A (PKA) signaling is altered in ACTH-independent macronodular adrenal hyperplasia similarly to adrenal tumors with 17q losses or *PRKAR1A* mutations (Bertherat et al., 2003; Bourdeau et al., 2006). Even more interesting was the finding that common adrenal lesions (i.e. adrenal adenomas) that did not harbor germline or somatic *GNAS*, *PRKAR1A*, *PDE11A*, and *PDE8B* mutations and were associated with ACTH-independent CS had functional abnormalities of the cAMP